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TECHNICAL REPORT 9116

# INTRAVENOUS (IV) FLUIDMAKER IV. A DISPOSABLE DEVICE FOR PREPARATION OF STERILE WATER FOR INJECTION IN A FIELD SETTING

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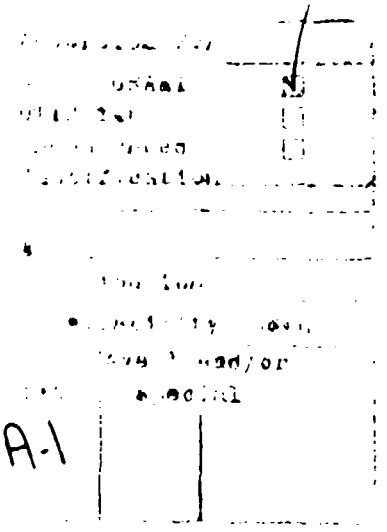

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A small, disposable system has been developed to prepare USP Sterile Water for Injection (WFI) from a pressurized potable source. The system comprises, in series, a standard laboratory strong acid/strong base ion exchange column, an activated carbon water purification filter, a fine particle filter, a 0.2 μm sterilizing filter and a Resuscitation Fluids Production System (REFLUPS) 18 L bag set, which is filled by means of a 19-port distribution head. This system is capable of generating sterile, pyrogen-free water at a fill rate of 0.5 L/min. Product resistivity did not consistently meet the 1.0 megohm limit corresponding to the USP standard for dissolved salts; a second, smaller, ion exchange cartridge will be required.

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## PREFACE

The contributions of Mr. Louie Martin and Mr. Leo Jenkins of USABRDL, who conceived, designed and constructed the 19-port indexed valve described in this study, are gratefully acknowledged.

## INTRODUCTION

The U.S. Army Institute of Surgical Research (USAISR) is interested in developing a disposable device to manufacture intravenous (IV) fluids from potable water for the resuscitation of burn patients.[1] The device must produce sterile, pyrogen-free water which can be introduced directly into sterile bags with sterile admixtures to make 1.0 L of Ringer's lactate, 1.0 L of 5 percent dextrose in water, or other parenterals suitable for IV infusion into humans. Approval by the Food and Drug Administration (FDA) will ultimately be required, but is not considered to be a part of this effort. Such a system should (1) occupy no more than 2 $ft^3$, (2) weigh no more than 10 kg, (3) have the capacity to produce 50 L of product (4) at a rate of 25 L/hr (5) from potable water at a pressure of no more than 30 psi (206 kpa).

In a previous study, two systems were devised for generating sterile, pyrogen-free water for injection (WFI) and were shown capable of producing WFI according to U.S. Pharmacopoeia (USP) standards.[2-4] Both systems utilize reverse osmosis, ion exchange, a solid matrix filter containing activated carbon and zeta adsorbent, a final 0.2 μm pore-size sterilizing filter and a device for transferring the WFI to an IV bag. The smaller system weighs approximately 1.5 kg and produces WFI at a rate of 1.0 L in 45-50 min; the larger weighs approximately 3.5 kg and produces 1.0 L of WFI in 5-6 min. The present study was initiated to address the higher production requirements of the USAISR.

## APPROACH AND RATIONALE

General performance requirements for the IV fluidmaker in terms of product quality are that it reduce dissolved inorganic and organic chemicals, including pyrogens, to very low levels; that it virtually eliminate residual suspended materials; and that it assure sterile transfer to an IV bag. Source water is long-term potable water as defined by TB MED 577[5] (Table 1). The target for the device in question (hereinafter the fluidmaker) is sterile WFI as defined by the USP XXII.[6] The USP manufacturing and purity criteria for Sterile Water for Injection are presented in Appendix A.

The high production requirement and the uncertainty of an external power supply rule out the use of reverse osmosis as in earlier studies and in the Resuscitation Fluids Production System (REFLUPS);[7] on the other hand, the 50-L limit and system disposability permit the use of laboratory ion exchange columns for total salt and heavy metals removal. The earlier studies showed that pyrogen removal and sterility can be achieved through use of a solid matrix activated carbon and zeta adsorbent filter of the kind used for household tapwater purification. The breadboard devised for the present study consisted, in series, of a strong acid/strong base mixed resin ion exchange column, carbon filter, fine particle filter and a 0.2 μm sterilizing filter. The product WFI was transferred through a closed system to an IV bag. For this operation we utilized an 18-bag transfer set originally developed for REFLUPS and a hand-operated, 19-port indexed valve directed fluid flow.

TABLE 1. FIELD POTABLE WATER STANDARDS[a]

| Constituent | Standard 7 days or less | Standard more than 7 days |
|---|---|---|
| PHYSICAL | | |
| Color | ----- | 50 units |
| Turbidity | reasonably clear | 5 NTU |
| CHEMICAL | | |
| Arsenic | 2.0 mg/L | 0.2 mg/L |
| Chloride | ----- | 600.0 mg/L |
| Cyanide | 20.0 mg/L | 2.0 mg/L |
| Magnesium | ----- | 150.0 mg/L |
| Sulfate | ----- | 400.0 mg/L |
| Total dissolved solids | ----- | 1500.0 mg/L |
| pH | ----- | 5.0-9.0 units |
| BACTERIOLOGICAL | | |
| Coliform | 1.0 per 100 mL | 1.0 per 100 mL |

a. Reference 5

## MATERIALS AND METHODS

WATER SUPPLY PUMP. A Masterflex® Model 7018 peristaltic pump and Masterflex® variable speed controller (Cole Parmer Instrument Co., Chicago, IL) were utilized for supplying challenge water to the system. System pressure was monitored by use of a 15 psi (100 kpa) pressure gauge. Tygon® tubing was utilized for the pump tubing as well as all other connections throughout the system.

ION EXCHANGE (IE) COLUMN. Barnstead/Thermolyne Corp. (Dubuque, IA) IE columns (cat. no. D8902) were utilized. These were off-the-shelf, ultrapure strong acid/strong base mixed resin units. The columns were each 16 inches (41 cm) long and contained approximately 1.4 kg of resin and with the addition of the plastic casing have a total weight of 1.7 kg.

WATER PURIFICATION FILTER. Seagull® filter cartridges, type RS1-SG (lot number 2765), and stainless steel cartridge housing (serial no. 098430) were acquired from General Ecology, Lionville, PA. The housing, with added stainless steel tubing fittings, weighed 725 g; each filter cartridge had a dry weight of 480 g.

FINE PARTICLE FILTERS. A Whatman® (Maidstone, Eng.) Gamma-12 filter unit, fitted with the grade 20 filter tube (0.2 μm rating) was used for test runs of Series 1. The tubes were autoclaved before use. For all subsequent tests, a Filterite (Timonium, MD) UIA4A spiral wound string filter cartridge was used. The stainless steel and brass filter cartridge housing (model 910562-000, type LM04B-1/2, serial HW) weighed 2.5 g with fittings, and the string filters each had a dry weight of 40 g. The spiral wound string filter

cartridges were autoclaved before use. Particle removal efficiency data for this filter are presented in Appendix B.

RECEIVER SET. The receiver sets (No. 15257), manufactured by Abbott Laboratories (North Chicago, IL) for Sterimatics Corporation (Bedford, MA) for REFLUPS, were used for all testing. Each sterile set consisted of 18 1-L IV bags (or 6 3-L bags or 18 0.5-L bags), a 7.5 cm diameter, 0.22 μm pore size sterilizing filter and a docking device, all contained in a plastic wrapper. The dry weight of an 18 1-L bag receiver set was 1.2 kg.

STERILIZING FILTER. As noted above, each receiver set contained its own sterilizing filter. MSI (Westboro, MA) nylon cameo filters of 0.22 μm pore size (cat. no. DD0200S15, lot no. 25004) were used only for limited testing.

BAGGING DEVICE. An indexed, 19-port valve was constructed from stainless steel and Teflon[R]. It weighed 1.54 kg and was designed to accept the Sterimatics REFLUPS receiver sets (Fig. 1).

SPRING SCALE. A spring scale, Ohaus Corp. (Florham Park, NJ) Model 8004-M0, 0-2000 g range, was used to determine filled bag weight for all testing. A 1-L bag was considered full when it contained 1000 g of liquid. The scale itself weighed 40 g.

CHALLENGE WATER. The challenge water was prepared by amending 400 gal (1500 L) of Fort Detrick tap water with 824 mg/liter of pulverized rock salt, bringing the TDS level to ca. 1000 mg/L and the conductivity to ca. 1200 μmho. The water was dechlorinated by vigorous mixing for two days, then was allowed to stand for two weeks to build up the level of naturally occurring bacteria and endotoxins. One 400 gal batch of challenge water was made up for the tests conducted during November 1990 (Series 1), the second was prepared in the same manner for testing during June 1991 (Series 2).

ANALYTICAL PROCEDURES. The Pyrogent Plus[R], Limulus Amebocyte Lysate (LAL) Gel Test Kit (product no. N284) used throughout this study was supplied by Whittaker Bioproducts, Inc., Walkersville, MD. A dilution series was employed for challenge water. Bacterial testing was conducted using BBL[R] prepared media (Becton Dickinson Microbiological Systems, Cockeysville, MD). For Series 1 tests custom ordered split media plates were used; one side of the plate consisted of TSA II[R] (Trypticase[R] soy agar with 5 percent Sheep Blood) and the other side contained MacConkey agar (MAC). A sample volume of 0.2 ml/side/plate was used for inoculations. Because no growth was observed on MAC media for any of the samples, no MAC agar data are reported. For tests conducted during Series 2, prepoured plates of TSA II[R] (catalog no. 21239/21261) were acquired from BBL and a 1-ml/plate volume was tested. All inoculated plates were incubated at 37°C for 48 hours before being evaluated. Conductivity was determined using a VWR Scientific (Media, PA) portable conductivity meter (Cat. No. 23198-014) for all Series 1 runs and Run 2, Series 2; a Presto-Tek[R] (Preston Scientific, Anaheim, CA) conductivity meter, model DP-03, was used for all other measurements.

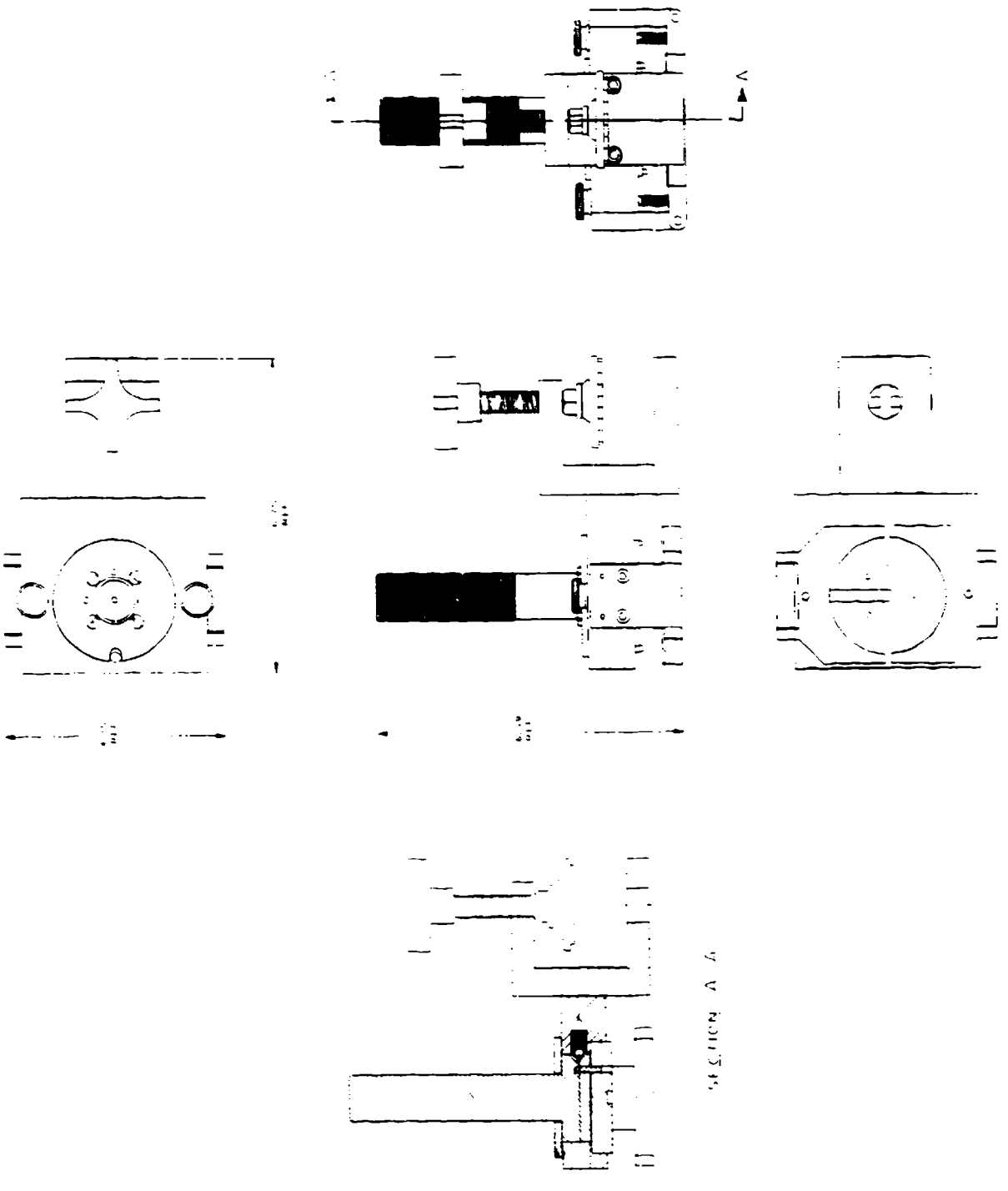

Figure 1. Bagging Device

TEST RUNS AND SAMPLING PROCEDURES. Each test of the system with uninterrupted product flow is defined as a run, whereas a series is a number of runs using the same challenge water. The first test series consisted of two runs. The system was configured as shown in Figures 2 and 3, with a Whatman® Gamma-12 filter unit for fine particle removal. For Series 1, Run 1 (Appendix Table C1) new filters and a new ion exchange column were installed in the housings, except for the Seagull® filter, which was omitted. Tygon® connections were cleaned with alcohol; the pump was activated and set at a rate of 500 mL/min. As the system filled the total system pressure registered 10-13 psi (69-89 kpa). After each bag was filled to 1 kg, the connecting fill hose was sealed with a pressure clamp in such a manner as to assure no cross contamination among bags when the receiver set was removed from the bagging device. After the bags were filled, the first receiver set (Set 1) was replaced, and the second set (Set 2) of bags was filled and removed in the same manner. Sets 1 and 2 were tested for sterility and conductivity. The unit was allowed to continue running with samples being collected by a clean catch method (no bags) following the ion exchange column; a total of 50 L was collected from all sets. Conductivity was the only parameter measured for the last 26 1-L samples collected (Set 3).

For Series 1, Run 2 (Appendix Table C2), all conditions were the same as the first run with the following exceptions: the Seagull® filter unit was installed and three different size bags were utilized due to a shortage of receiver sets. Three sets were collected for this run: 6 3-L bags, 9 0.5-L bags and 16 1-L bags. The run was terminated after only 16 bags were filled in the last set because of a drop in flow resulting from plugging of the fine particle filter. Endotoxin and bacteriological plate tests were performed for all samples collected during this run.

The Filterite® fine particle filter was used for all Series 2 tests rather than the Whatman® filter. For Series 2, Run 1 (Appendix Table C3), the bagging device and the receiver set were removed as a unit after all bags were filled. This obviated the use of pressure clamps (as in Series 1) to prevent cross contamination of samples. Following removal of the bag set, the fluidmaker was allowed to continue running and conductivity was monitored until breakthrough of the ion exchange column. For Series 2, Runs 2 and 3 (Appendix Tables C4 and C5, resp.), all filters were replaced and all connections were again washed with alcohol. The same conditions existed and the same procedures were followed as in Run 1 except that the primer bag (a 500 mL IV bag which catches the first flow and is discarded) was removed and two 1-L samples were collected before filling commenced. For Series 2, Run 4 (Appendix Table C6), the primer bag was removed and six successive 0.5 L samples were collected by clean catch. The IV bags were not filled, and sterility tests were not performed.

Two supplemental runs (Appendix Tables D1 and D2) were carried out to investigate conductivity leached from the Seagull® RS-1SG filter. All components of the fluidmaker were removed except the pump, ion exchange column and Seagull® filter. A sampling port was inserted between the ion exchange column and the filter. Fort Detrick tapwater was passed through the abbreviated fluidmaker at 0.5 L/min and regular observations of conductivity were made.

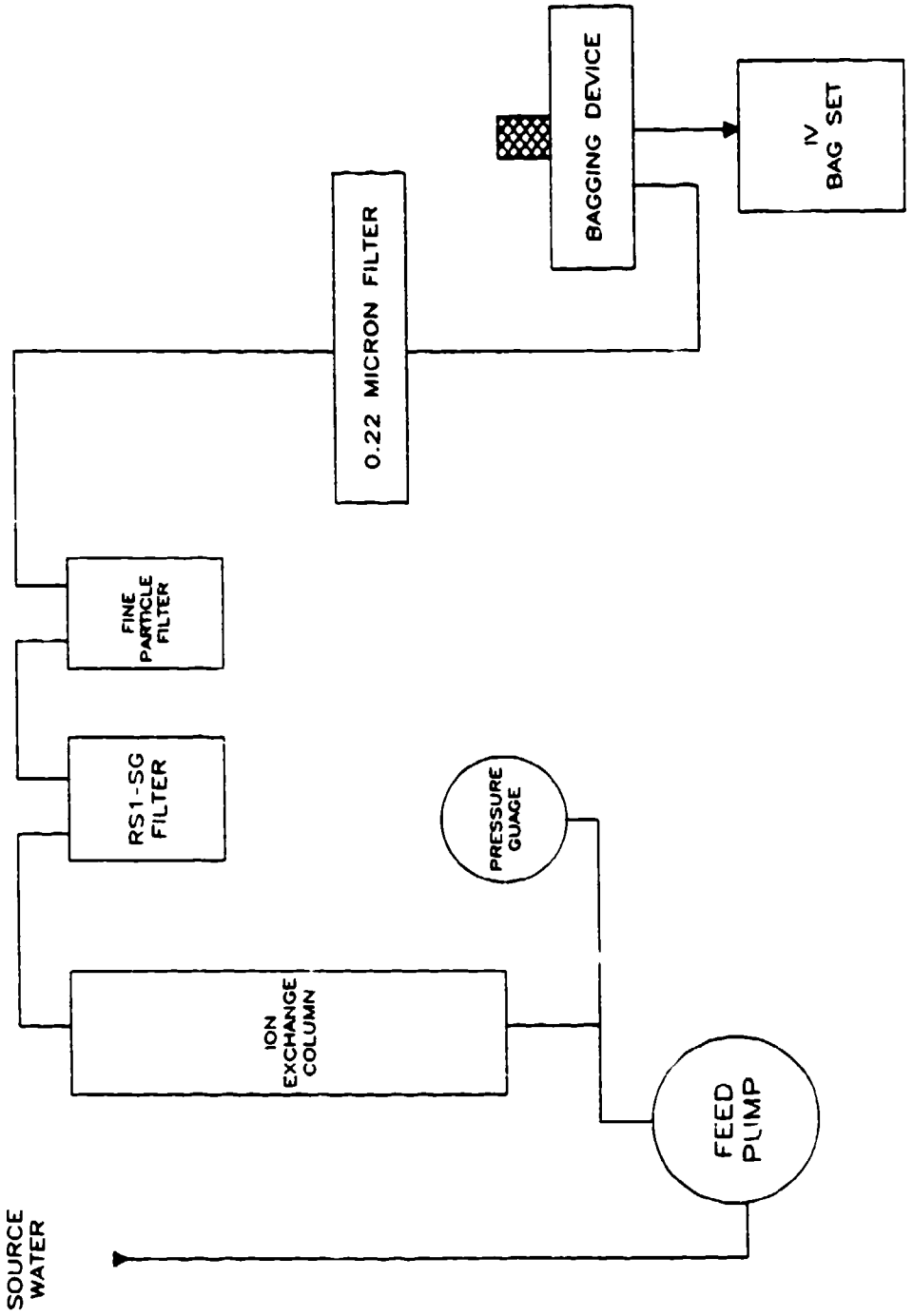


Figure 2. Schematic of IV Fluidmaker

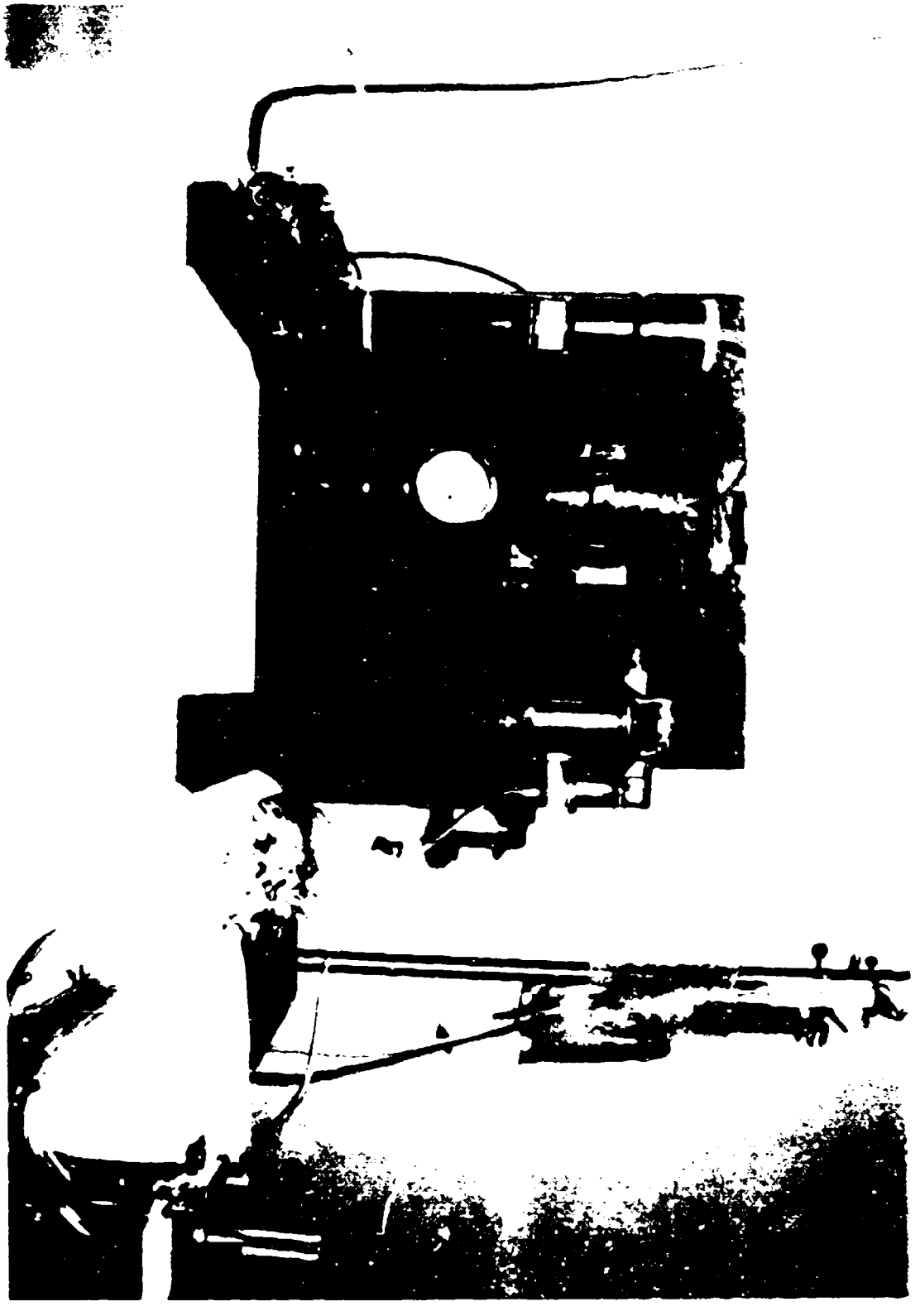

Figure 3. Test Stand

All receiver sets were processed in the same manner (except for the supplemental runs). The receiver sets were never removed from the bagging device or, in the case of Series 1 testing, the clamps were not removed. In order, each bag filler hose was first wiped with alcohol and then cut with flamed stainless steel scissors to separate the individual bag from the receiver set. The samples for bacteriological and LAL testing were collected in sterile Falcon® 2027 screw cap tubes using a clean catch method. Another sample was then collected for conductivity testing.

## RESULTS AND DISCUSSION

The breadboard test system meets all the initial requirements,[1] having produced sterile, pyrogen-free water from potable water at a rate of 0.5 L/min at a feed pressure of one atmosphere or less. The challenge water contained levels of bacteria [≥11,000 colony forming units (cfu)/mL] and pyrogens [≥60 endotoxin units (eu)/mL] much higher than would be expected for any potable source. The combined weight of all components is less than 10 kg, and the complete device with three bag sets (total capacity 54 L) occupies no more than two cubic feet, as required.[1] Strengths and deficiencies of individual components will be discussed in order.

ION EXCHANGE (IE) COLUMN. The purpose of the IE column is to reduce dissolved inorganic components of the feed water to acceptable levels. Collectively, these levels can be approximated in terms of conductivity; water meeting USP XXII standards[6] should have conductivity no greater than ca. 1 μmho (or resistivity no less than 1 megohm). The challenge water for most of the tests had a total dissolved solids content (TDS) of ca. 1000 mg/L and conductivity of 1250±50 μmho. The Barnstead IE cartridges employed did not consistently meet the conductivity limit with this challenge (Appendix Table C1). However, the significant breakthrough did not occur until total product volume had exceeded 60 L or more. In two successive tests with a weaker challenge, a minor breakthrough occurred at about 8 L. There is a second source of conductivity in the system that will have to be addressed, but we conclude that the Barnstead cartridge is suitable for initial reduction of conductivity.

WATER PURIFICATION FILTER. The function of the solid matrix water purification filter is removal of endotoxins and other organic materials. The Seagull® IV RS1-SG filter reduced the endotoxins to below the LAL detection limit (0.06 eu/mL) and well below the USP XXII standard (0.25 eu/mL). However, endotoxin breakthrough at the detection limit occurred at 38 L of product from a challenge greater than 100 eu/mL. To assure 54 L of pyrogen-free water it may be necessary to install a new filter for each 18 L bag set. An alternative would be to substitute the larger Seagull® IV RS2-SG filter (700 g vs 450 g) and X2 housing (1.2 kg vs 725 g), which should allow depyrogenation of 54 L with a single filter. Combined weights would be about the same. It was observed that there is significant leaching of conductive materials from the RS1-SG filter (Tables D1 and D2), a problem that did not arise with the smaller First Need® filters employed in our original study.[2] We have not identified these materials, but the problem should be correctable by means of a second, much smaller, ion exchange column in series, preferably

one with medical grade resin. The size and shape of this column would be dictated by flow considerations rather than exchange capacity, which would not in any event exceed 1 g as sodium chloride.

FINE PARTICLE FILTER. The function of the fine particle filter is to protect the sterilizing filter, part of the receiver set, from blockage by small particles shed by the Seagull® filter. A Whatman® tube filter rated at 0.2 μm pore size was used for test runs of Series 1. This filter protected the sterilizing filter, but plugged after 43 L of product water (Appendix Table C2). Use of the Whatman® system would require changing filter tubes after each 18 L bag set. For later runs, a Filterite® wound string cartridge was used. This system protected the sterilizing filter and showed no signs of restricted flow for more than 100 L of product (Appendix Tables C3-C5). The weight of the stainless steel and brass housing, 2 5 kg, is a disadvantage, but we have been informed that a lighter weight plastic housing is available. A second disadvantage is the spin finish, applied to the filter during manufacture, which comprises a mixture of calcium stearate, polyoxyethylene, fatty acids and fatty esters. All are approved by the Food and Drug Administration, according to the manufacturer, but they give a false positive LAL indication for the filtrate, as shown in Appendix Tables C3-C6, thus interfering with the test for endotoxins. The manufacturer has informed us that a prewashed medical grade filter, identical in all other respects, is available.

BAGGING DEVICE. The bagging device constructed for this project, an indexed, manually operated 19-port valve, accepted the REFLUPS receiver sets (Figure 1). Fluid transfer to individual IV bags was readily achieved, and no leakage occurred. In the first run of the second series, two bags showed bacterial contamination at a level of 1-2 cfu/mL (Appendix Table C3); otherwise, all samples collected were sterile, and we believe that the nonsterile samples were artifacts of the sampling procedure. Two issues have yet to be addressed with respect to the receiver sets: a provision for incorporating parenteral concentrate and a means for sealing individual IV bags must be devised. For sealing the bags, slotted plastic tags (Figure 4) could be incorporated during manufacture of the receiver sets, or a battery-operated heat sealer could be included in the fluidmaker package. The Johnson Space Center has addressed the question of parenteral concentrates,[8] but we are unaware of current progress.

## SUMMARY AND RECOMMENDATIONS

A water purifier consisting of an ion exchange column, a solid matrix carbon filter, a fine particle filter, a mechanical bagging device and a REFLUPS receiver set, has produced sterile, pyrogen-free water at a rate of 0.5 L/min from a low-pressure potable source. The prototype package is projected to weigh no more than 10 kg and occupy no more than 2 $ft^3$ of space. A considerable developmental effort remains to convert the breadboard system tested to a prototype fluidmaker, namely:

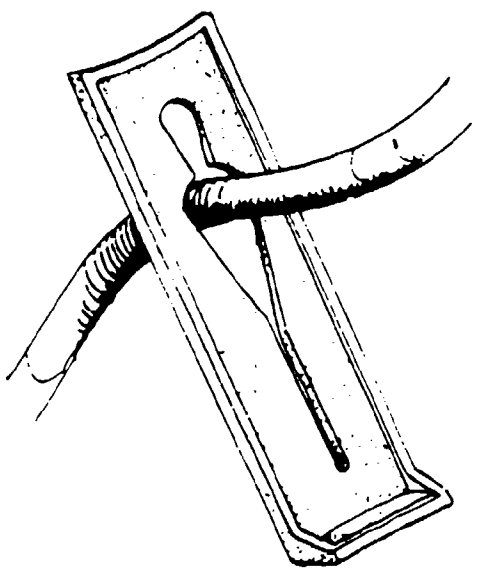

Figure 4. Device for Sealing IV Bags

1. The ability of the Seagull[R] IV RS2-SG water purification filter to consistently depyrogenate at least 54 L of challenge water should be tested.

2. A high-flow, low capacity IE column, with medical grade resin, is needed to deionize the weakly conductive flow from the water purification filter.

3. The acceptability of the plastic housing for the Filterite[R] fine particle filter should be investigated. This substitution could reduce the system weight by 2 kg.

4. Pre-washed Filterite[R] fine particle filters should be tested to assure that the filtrate does not give false positive LAL endotoxin tests.

5. A suitable method for sealing the IV bags in the field should be devised.

6. A method for introducing parenteral concentrates must be developed.

The final configuration for the prototype fluidmaker package involves training and doctrine issues beyond the scope of this report. However, we suggest that the best system will involve minimum attention other than filling of bags. Thus, the requirement for changing filters between receiver sets would not be desirable. We further suggest that the bagging device should be recovered after use; this device does not come in contact with the product water, does not require sterilizing, and is the most expensive item in the system.

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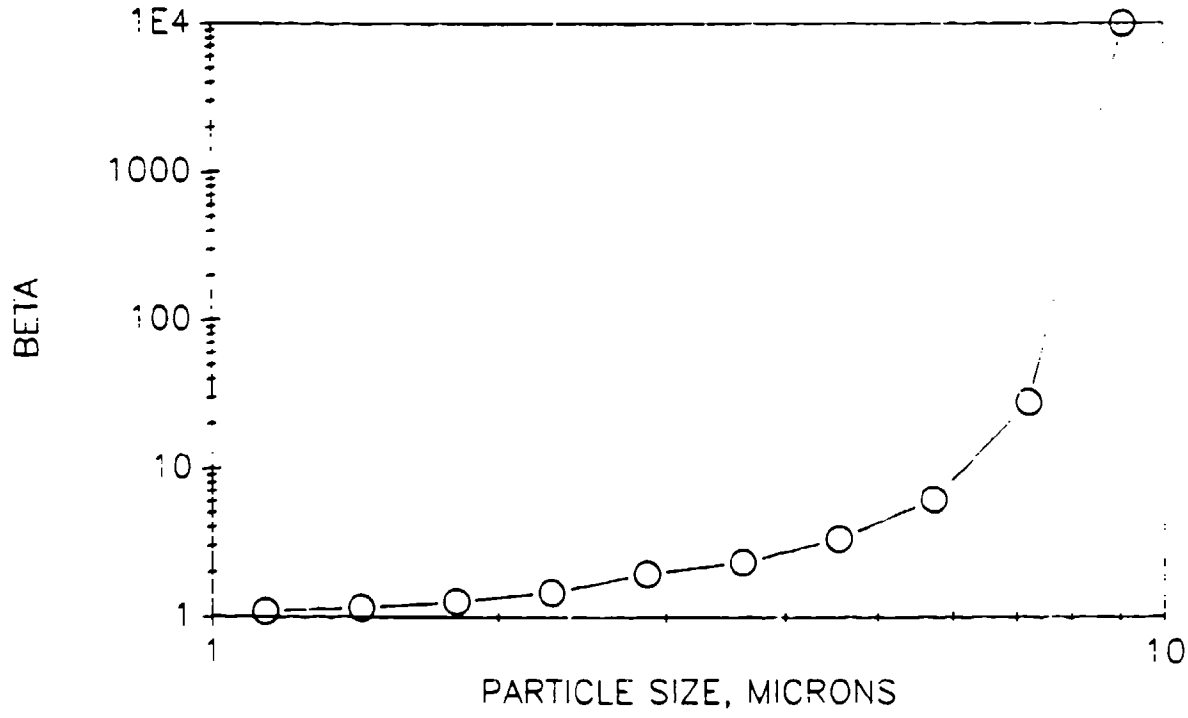
## APPENDIX A: STERILE WATER FOR INJECTION[6]

Sterile Water for Injection is Water for Injection sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

**Packaging and storage** -- Preserve in single-dose containers, preferably of Type I or Type II glass, of not larger than 1-liter size.
**Labeling** -- Label it to indicate that no antimicrobial or other substance has been added, and that it is not suitable for intravascular injection without its first having been made appropriately isotonic by the addition of a suitable solute.
**Reference standard** -- USP Endotoxin Reference Standard.
**Bacterial endotoxins** -- When tested as directed under Bacterial Endotoxins Test <85>, the USP Endotoxin RS being used, it contains not more than 0.25 USP Endotoxin Unit per mL.
**Sterility** -- It meets the requirements under Sterility Tests <71>.
**Ammonia** -- For Sterile Water for Injection in glass containers holding a volume up to 50 mL, dilute 50 mL with 50 mL of High-purity Water (see Reagents under Containers <661>), and use this dilution as the test solution; where larger volumes are held, use 100 mL of Sterile Water for Injection as the test solution. To 100 mL of the test solution add 2 mL of mercuric-potassium iodide TS: any yellow color produced immediately is not darker than that of a control containing 30 $\mu$g of added $NH_3$ in High-purity Water (see Reagents under Containers <661>)(0.6 ppm for Sterile Water for Injection packaged in volumes up to 50 mL in containers; 0.3 ppm for larger volumes).
**Chloride** -- To 20 mL in a color-comparison tube add 5 drops of nitric acid and 1 mL of silver nitrate TS, and gently mix: any turbidity formed within 10 minutes is not greater than that produced in a similarly treated control consisting of 20 mL of High-purity Water (see under Reagents in Containers <661>) containing 10 $\mu$g of Cl (0.5 ppm), viewed downward over a dark surface with light entering the tubes from the sides.
**Oxidizable substances** -- To 100 mL add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Water for Injection in containers holding a volume up to 50 mL, add 0.4 mL of 0.1 N potassium permanganate, and boil for 5 minutes; for larger volumes, add 0.2 mL of 0.1 N potassium permanganate, and boil for 5 minutes: the pink color does not completely disappear.
**Total solids** -- Proceed as directed in the test for Total solids under Purified Water. The following limits apply for Sterile Water for Injection in glass containers holding up to 30 mL, 0.004%; from 30 mL up to 100 mL, 0.003%; and for larger volumes, 0.002%.
**Other requirements** -- It meets the requirements of the tests for pH, Sulfate, Calcium, Carbon dioxide, and Heavy metals under Purified Water.

## APPENDIX B: FINE PARTICLE FILTER EFFICIENCY DATA




The fraction of particles of any diameter removed by the Filterite$^{R}$ U01A4S filter is given by $(\beta-1)/\beta$. Data were provided through the courtesy of the Customer Services Laboratory of Memtec America Corporation.

## APPENDIX C: TEST DATA FOR PRODUCTION RUNS

TABLE C1. SERIES 1, RUN 1

| Sample | cfu/mL | LAL | Conductivity μmhos | Cumulative volume, L |
|---|---|---|---|---|
| Feed undiluted | TNTC[a] | | 1279 | |
| Feed $1X10^{-1}$ | TNTC | + | | |
| Feed $1X10^{-2}$ | 440 | + | | |
| Feed $1X10^{-3}$ | 115 | + | | |
| Feed $1X10^{-4}$ | 15 | + | | |
| Feed $1X10^{-5}$ | 0 | - | | |
| Set 1: 500-mL bags[b] | | | | |
| 1 | 0 | | .15 | 1 |
| 2 | 0 | | .18 | 2 |
| 3 | 0 | | 1.02 | 3 |
| 4 | 0 | | 1.04 | 4 |
| 5 | 0 | | .62 | 5 |
| 6 | 0 | | .78 | 6 |
| 7 | 0 | | .68 | 7 |
| 8 | 0 | | .91 | 8 |
| 9 | 0 | | .81 | 9 |
| Set 2: 1000-mL bags | | | | |
| 10 | 0 | | .88 | 10 |
| 11 | 0 | | .81 | 11 |
| 12 | 0 | | .78 | 12 |
| 13 | 0 | | .70 | 13 |
| 14 | 0 | | .74 | 14 |
| 15 | 0 | | .69 | 15 |
| 16 | 0 | | .75 | 16 |
| 17 | 0 | | .72 | 17 |
| 18 | 0 | | .72 | 18 |
| 19 | 0 | | .74 | 19 |
| 20 | 0 | | .72 | 20 |
| 21 | 0 | | .74 | 21 |
| 22 | 0 | | .74 | 22 |
| 23 | 0 | | .76 | 23 |
| 24 | 0 | | .78 | 24 |
| Set 3: No bags[c] | | | | |
| 25 | | | 1.07 | 25 |
| 26 | | | 1.07 | 26 |
| 27 | | | 1.11 | 27 |
| 28 | | | 1.12 | 28 |
| 29 | | | 1.11 | 29 |
| 30 | | | 1.11 | 30 |
| 31 | | | 1.16 | 31 |
| 32 | | | 1.13 | 32 |

TABLE C1. SERIES 1, RUN 1, CONT.

| Sample | cfu/mL | LAL | Conductivity μmhos | Cumulative volume, L |
|---|---|---|---|---|
| 33 | | | 1.17 | 33 |
| 34 | | | 1.13 | 34 |
| 35 | | | 1.10 | 35 |
| 36 | | | 1.14 | 36 |
| 37 | | | 1.20 | 37 |
| 38 | | | 1.20 | 38 |
| 39 | | | 1.18 | 39 |
| 40 | | | 1.16 | 40 |
| 41 | | | 1.18 | 41 |
| 42 | | | 1.28 | 42 |
| 43 | | | 1.14 | 43 |
| 44 | | | 1.14 | 44 |
| 45 | | | 1.14 | 45 |
| 46 | | | 1.13 | 46 |
| 47 | | | 1.14 | 47 |
| 48 | | | 1.22 | 48 |
| 49 | | | 1.08 | 49 |
| 50 | | | 1.06 | 50 |

a. TNTC = too numerous to count.
b. Two 500-mL bags per sample.
c. Only conductivity measured for Set 3 samples.

TABLE C2. SERIES 1, RUN 2

| Sample | cfu/mL | LAL | Conductivity μmhos | Cumulative volume, L |
|---|---|---|---|---|
| Set 1: 3 liter bags | | | | |
| 1 | 0 | - | 30.8 | 3 |
| 2 | 0 | - | 30.8 | 6 |
| 3 | 0 | - | 22.6 | 9 |
| 4 | 0 | - | 4.5 | 12 |
| 5 | 0 | - | 8.7 | 15 |
| 6 | 0 | - | 7.4 | 18 |
| Set 2: 500-mL bags[a] | | | | |
| 7 | 0 | - | 4.0 | 19 |
| 8 | 0 | - | 3.4 | 20 |
| 9 | 0 | - | 2.9 | 21 |
| 10 | 0 | - | 2.4 | 22 |
| 11 | 0 | - | 2.2 | 23 |
| 12 | 0 | - | 1.9 | 24 |
| 13 | 0 | - | 1.9 | 25 |
| 14 | 0 | - | 1.6 | 26 |
| 15 | 0 | - | 1.4 | 27 |
| Set 3: 1 liter bags | | | | |
| 16 | 0 | - | 1.7 | 28 |
| 17 | 0 | - | 1.6 | 29 |
| 18 | 0 | - | 1.7 | 30 |
| 19 | 0 | - | 1.6 | 31 |
| 20 | 0 | - | 1.5 | 32 |
| 21 | 0 | - | 1.2 | 33 |
| 22 | 0 | - | 1.5 | 34 |
| 23 | 0 | - | 1.4 | 35 |
| 24 | 0 | - | 1.3 | 36 |
| 25 | 0 | - | 1.2 | 37 |
| 26 | 0 | + | 1.2 | 38 |
| 27 | 0 | + | 1.2 | 39 |
| 28 | 0 | + | 1.2 | 40 |
| 29 | 0 | + | 1.2 | 41 |
| 30 | 0 | + | 1.4 | 42 |
| 31[b] | 0 | + | 1.4 | 43 |

a. Two 500-mL bags per sample.
b. Fine particle filter plugged; last 2 samples not collected.

TABLE C3. SERIES 2, RUN 1

| Sample | cfu/mL | LAL | Conductivity µmho | Cumulative volume, L |
|---|---|---|---|---|
| Sterile $H_2O$ | 0 | - | 1 | |
| Feed undiluted | TNTC[a] | + | 1200 | |
| Feed $1X10^{-1}$ | TNTC | + | ---- | |
| Feed $1X10^{-2}$ | 45 | + | ---- | |
| Feed $1X10^{-3}$ | 11 | + | ---- | |
| Feed $1X10^{-4}$ | 0 | - | ---- | |
| Set 1: 1000 mL bags | | | | |
| 1 | 0, 2[b] | + | 40 | 1 |
| 2 | 0, 0 | + | 35 | 2 |
| 3 | 0, 0 | - | 21 | 3 |
| 4 | 0, 0 | - | 17 | 4 |
| 5 | 0, 0 | - | 13 | 5 |
| 6 | 0, 0 | - | 12 | 6 |
| 7 | 0, 0 | - | 10 | 7 |
| 8 | 0, 0 | - | 8 | 8 |
| 9 | 0, 0 | - | 7 | 9 |
| 10 | 0, 0 | - | 6 | 10 |
| 11 | 0, 0 | - | 7 | 11 |
| 12 | 0, 0 | - | 6 | 12 |
| 13 | 0, 1 | - | 5 | 13 |
| 14 | 0, 0 | - | 5 | 14 |
| 15 | 0, 0 | - | 5 | 15 |
| 16 | 0, 0 | - | 5 | 16 |
| 17 | 0, 0 | - | 4 | 17 |
| 18 | 0, 0 | - | 4 | 18 |
| 19[b] | | | 6 | 19 |
| 20 | | | 6 | 20 |
| 21 | | | 4 | 21 |
| 22 | | | 4 | 22 |
| 23 | | | 4 | 23 |
| 24 | | | 3 | 24 |
| 25 | | | 2 | 25 |
| 26-58 | | | 3-4 | 58 |
| 70 | | | 5 | 70 |
| 78 | | | 15 | 78 |
| 83 | | | 42 | 83 |
| 88 | | | 90 | 88 |
| 93 | | | 160 | 93 |
| 98 | | | 200 | 98 |
| 103 | | | 520 | 103 |
| 108 | | | 1000 | 108 |

a. Duplicate samples. b. TNTC = too numerous to count.
c. Only conductivity measured for samples 19-108; samples not bagged.

TABLE C4. SERIES 2, RUN 2

| Sample | cfu/mL | LAL | Conductivity µmho | Cumulative volume, L |
|---|---|---|---|---|
| Sterile H20 | 0 | - | 1 | |
| Before Bagging | | | | |
| Liter 1 | 0 | + | 34.3 | 1 |
| Liter 2 | 0 | - | 19.4 | 2 |
| Set 1: 1-L bags | | | | |
| 1 | 0 | - | 17.8 | 3 |
| 2 | 0 | - | 17.5 | 4 |
| 3 | 0 | - | 13.2 | 5 |
| 4 | 0 | - | 2.1 | 6 |
| 5 | 0 | - | 2.1 | 7 |
| 6 | 0 | - | 3.2 | 8 |
| 7 | 0 | - | 1.1 | 9 |
| 8 | 0 | - | 4.0 | 10 |
| 9 | 0 | - | 3.2 | 11 |
| 10 | 0 | - | 2.1 | 12 |
| 11 | 0 | - | 3.2 | 13 |
| 12 | 0 | - | 2.0 | 14 |
| 13 | 0 | - | 1.1 | 15 |
| 14 | 0 | - | 1.6 | 16 |
| 15 | 0 | - | 3.5 | 17 |
| 16 | 0 | - | 2.2 | 18 |
| 17 | 0 | - | 2.3 | 19 |
| 18[a] | 0 | - | 2.2 | 20 |
| 38 | | | 2 | 40 |
| 58 | | | 2 | 50 |
| 78 | | | 4 | 60 |
| 88 | | | 11 | 90 |
| 93 | | | 18 | 95 |
| 98 | | | 48 | 100 |
| 102 | | | 100 | 104 |
| 105 | | | 250 | 107 |
| 109 | | | 440 | 111 |
| 111 | | | 1000 | 113 |

a. Only conductivity measured for samples 18-111; samples not bagged.

TABLE C5. SERIES 2, RUN 3

| Sample | cfu/mL | LAL | Conductivity µmho | Cumulative volume, L |
|---|---|---|---|---|
| Sterile $H_2O$ | 0, 0[a] | - | 1 | |
| Feed undiluted | TNTC[b] | + | 1250 | |
| Feed $1X10^{-1}$ | TNTC | + | ---- | |
| Feed $1X10^{-2}$ | 68 | + | ---- | |
| Feed $1X10^{-3}$ | 22 | + | ---- | |
| Feed $1X10^{-4}$ | 0 | - | ---- | |
| Before Bagging | | | | |
| Liter 1 | 0 | + | 47 | 1 |
| Liter 2 | 0 | + | 28 | 2 |
| Set 1: 1-L bags | | | | |
| 1 | 0 | - | 24 | 3 |
| 2 | 0 | - | 16 | 4 |
| 3 | 0 | - | 15 | 5 |
| 4 | 0 | - | 12 | 6 |
| 5 | 0 | - | 11 | 7 |
| 6 | 0 | - | 9 | 8 |
| 7 | 0 | - | 8 | 9 |
| 8 | 0 | - | 6 | 10 |
| 9 | 0 | - | 7 | 11 |
| 10 | 0 | - | 6 | 12 |
| 11 | 0 | - | 5 | 13 |
| 12 | 0 | - | 3 | 14 |
| 13 | 0 | - | 3 | 15 |
| 14 | 0 | - | 4 | 16 |
| 15 | 0 | - | 3 | 17 |
| 16 | 0 | - | 3 | 18 |
| 17 | 0 | - | 3 | 19 |
| 18[b] | 0 | - | 3 | 20 |
| 38 | | | <1 | 40 |
| 58 | | | 2 | 60 |
| 68 | | | 200 | 70 |
| 80 | | | 700 | 82 |
| 90 | | | 1000 | 92 |

a. Duplicate samples.
b. TNTC = too numerous to count.
c. Only conductivity measured for samples 38-90; samples not bagged.

TABLE C6. SERIES 2, RUN 4

| Sample | Dilution | LAL | Conductivity μmho |
|---|---|---|---|
| Feed | undiluted | + | 1300 |
| Feed | $1X10^{-1}$ | + | ---- |
| Feed | $1X10^{-2}$ | + | ---- |
| Feed | $1X10^{-3}$ | + | ---- |
| Feed | $1X10^{-4}$ | - | ---- |
| Product | | | |
| 0.5 L | undiluted | + | 100 |
| | 1:1 | + | ---- |
| | 1:3 | + | ---- |
| | 1:5 | - | ---- |
| 1.0 L | undiluted | + | 58 |
| | 1:1 | + | ---- |
| | 1:3 | - | ---- |
| | 1:5 | - | ---- |
| 1.5 L | undiluted | + | 43 |
| | 1:1 | - | ---- |
| | 1:3 | - | ---- |
| | 1:5 | - | ---- |
| 2.0 L | undiluted | + | 35 |
| | 1:1 | - | ---- |
| | 1:3 | - | ---- |
| | 1:5 | - | ---- |
| 2.5 L | undiluted | + | 30 |
| | 1:1 | - | ---- |
| | 1:3 | - | ---- |
| | 1:5 | - | ---- |
| 3.0 L | undiluted | - | 25 |
| | 1:1 | - | ---- |
| | 1:3 | - | ---- |
| | 1:5 | - | ---- |

## APPENDIX D: LEACH TESTS FOR WATER PURIFICATION FILTERS

TABLE D1. CONDUCTIVITY CONTRIBUTIONS FROM IE AND RS1-SG, RUN 1

| Volume collected, L | Conductivity, IE[a] μmho | Conductivity, RS1-SG[b] μmho |
|---|---|---|
| 0 | 1.0 | |
| 1 | 0.5 | 100 |
| 2 | 0.3 | 40 |
| 3 | 0.3 | 23 |
| 4 | 1.0 | 20 |
| 5 | 1.0 | 16 |
| 6 | 0.3 | 12.5 |
| 7 | 0.2 | 10.2 |
| 8 | 0.3 | 8.5 |
| 9 | 1.0 | 7.5 |
| 10[c] | 6.0 | 15 |
| 11 | 7.0 | 19 |
| 12 | 6.0 | 14 |
| 13 | 5.0 | 10 |
| 14 | 5.0 | 9 |
| 15 | 4.5 | 8 |
| 16 | 4.5 | 8 |
| 17 | 5.0 | 7.5 |
| 18 | 6.5 | 7 |
| 20 | 6.0 | 7 |
| 22 | 6.0 | 7 |
| 26 | 6.0 | 7 |
| 30 | 6.0 | 7 |
| 35 | 6.0 | 7.5 |
| 40 | 6.0 | 7 |

a. Feed conductivity 500 ± 50 μmho.
b. Includes contributions from IE column + RS1-SG.
c. The peristaltic pump failed during collection of the 10th liter; the system was down for about 30 min.

TABLE D2. CONDUCTIVITY CONTRIBUTIONS FROM IE AND RS1-SG, RUN 2

| Volume collected, L | Conductivity, IE[a] μmho | Conductivity, RS1-SG[b] μmho |
|---|---|---|
| 0 | 0.2 | |
| 1 | 0.5 | 90 |
| 2 | 0.6 | |
| 3 | 1.0 | 26 |
| 4 | 0.5 | 21 |
| 5 | 0.5 | 16 |
| 6 | 0.3 | 15 |
| 7 | 0.6 | 12 |
| 8 | 0.5 | 9 |
| 9 | 1.0 | 9 |
| 10 | 1.5 | 8.5 |
| 11 | 2.0 | 8 |
| 12 | 2.5 | 8 |
| 13 | 3.0 | 8 |
| 14 | 3.5 | 8 |
| 15 | 4.0 | 8 |
| 16 | 4.5 | 8 |
| 17 | 4.5 | 8 |
| 18 | 5.0 | 8 |
| 19 | 5.0 | 8 |
| 20 | 5.0 | 7.5 |
| 25 | 5.0 | 7.5 |
| 30 | 5.5 | 7.5 |
| 35 | 5.5 | 7.3 |
| 40 | 5.5 | 7.0 |

a. Feed conductivity 500 ± 50 μmho.
b. Includes contributions from IE column + RS1-SG.

## APPENDIX E: LAL KIT SENSITIVITY TESTING

TABLE E1. LAL TEST SENSITIVITY

| eu/mL | LAL Results |
|---|---|
| 0.50 | + |
| 0.25 | + |
| 0.12 | + |
| 0.06 | + |
| 0.03 | - |

## APPENDIX F: GLOSSARY OF TERMS

| | |
|---|---|
| cfu | colony forming units |
| eu | endotoxin units |
| FDA | Food and Drug Administration |
| IE | ion exchange |
| IV | intravenous, intravascular |
| kPa | kilopascal |
| LAL | Limulus amebocyte lysate |
| MAC | MacConkey agar |
| NTU | nephelometric turbidity units |
| psi | pounds per square inch |
| REFLUPS | resuscitation fluids production system |
| TDS | total dissolved solids |
| TSA | Trypticase® soy agar |
| TNTC | too numerous to count |
| USABRDL | U.S. Army Biomedical Research and Development Laboratory |
| USAISR | U.S. Army Institute of Surgical Research |
| USP | U.S. Pharmacopeia |
| WFI | water for injection |

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